

# Cryopreserving and deglycerolizing sickle cell trait red blood cell components using an automated cell-processing system

R.J. ACKLEY, A.H. LEE-STROKA, B.J. BRYANT, D.F. STRONCEK, AND K.M. BYRNE

RBC components with rare phenotypes are sometimes required for patients with sickle cell disease, and these rare components can often be found among donors with sickle cell trait. Cryopreserving RBC components from sickle cell trait donors requires a modified deglycerolization method to preserve the integrity of the RBCs. This study evaluated the feasibility of using an automated cell-processing system to cryopreserve and deglycerolize sickle cell trait donor RBC components. CP2D/AS-3 RBC components were collected from three donors with sickle cell trait. Each component was processed with an automated cell-processing system (ACP 215, Haemonetics Corp., Braintree, MA) and cryopreserved within 6 days of collection. The components were stored at  $-65^{\circ}\text{C}$  or less for at least 2 days and were deglycerolized using the automated cell-processing system's standard procedure. Before cryopreservation and after deglycerolization, several variables were measured. Deglycerolization resulted in recovery of 43.0, 76.5, and 67.5 percent of RBCs from the three sickle-cell-trait donor components compared with 80 percent or greater for all six control components. A small, dark red, jelly-like mass was noted in the bowl of the disposable set after deglycerolization of each of the three RBC sickle cell trait components. The osmolalities of all three sickle cell trait components were less than 400 mOsm/kg, but only one of the three was acceptable for a 14-day outdate. Freezing and deglycerolization of sickle cell trait donor RBC components with the automated cell-processing system resulted in recovery of some RBCs, but a decrease in RBC recovery was problematic. Modifications of the procedure are needed for processing sickle cell trait donor RBC components. *Immunohematology* 2008;24:107-111.

**Key Words:** glycerolization, deglycerolization, sickle cell trait, cryopreservation

Patients with sickle cell disease (SCD) often require RBC transfusions throughout their lifetimes. These patients are exposed to blood from multiple

donors and have an increased chance of forming alloantibodies. According to one study, transfused patients with SCD have up to a 29 percent chance of becoming alloimmunized.<sup>1</sup> This immunization occurs because the majority of RBC transfusions are obtained from Caucasian donors and subsequently result in exposure to major differences in antigens.<sup>2</sup>

Because of the differing hospital protocols concerning the matching of RBC blood group phenotypes of patients with SCD and RBC component donors, these patients may receive RBC components that have only been matched for ABO and D blood group antigens. Osby and Shulman<sup>3</sup> report results of a survey that indicate 62.9 percent of hospitals crossmatch only ABO/D-compatible blood whereas 27.9 percent determine the patient's baseline phenotype for commonly immunogenic antigens and crossmatch ABO/D-compatible and phenotypically matched RBC units. Alternatively, 9.2 percent of hospitals report that the patient's baseline phenotype for common antigens is determined, but crossmatch only ABO/D-compatible RBC units initially.<sup>3</sup> After an antibody is made, all subsequent RBC units transfused are antigen matched. Initiation of transfusion of phenotypically matched RBC units only after immunization occurs is based on the observation that some patients develop alloantibodies after relatively few RBC transfusions and are referred to as "responders" whereas others may be "nonresponders" who may not make antibodies even after multiple

transfusions with RBCs matched only for ABO and D blood group antigens.<sup>1</sup>

The risk of alloimmunization of patients with SCD may make it difficult to find phenotypically compatible RBC components for transfusion. Some patients need RBC components from donors with unusual phenotypes that can only be found in repositories of cryopreserved RBC components with rare phenotypes. The probability of finding compatible components for patients with SCD is higher among donors who are African or of African descent, who are more likely to have similar RBC phenotypes.<sup>4</sup>

Blood donors who are likely to have RBCs phenotypically compatible with those of patients with SCD are also at risk to be heterozygous for the gene encoding hemoglobin S (HbS) and thus be HbS/HbA. The donor population that expresses heterozygosity for the gene encoding HbS has sickle cell trait, but is healthy. However, collecting and preparing RBC components from donors with sickle cell trait presents two difficulties. The first is filter failure, which can be defined as the inability to remove a sufficient number of leukocytes, and the second is the clotting of the blood inside the filter. Neither of these outcomes is inevitable, but one study found that approximately half of the RBC components collected from people with sickle trait occlude WBC-reduction filters, one quarter pass completely through the filter but the quantity of WBCs remaining exceeds criteria for WBC reduction, and only one quarter are effectively filtered.<sup>5</sup> Polymerization of HbS during the collection and processing of the blood is responsible for the occlusion of leukocyte-reduction filters, but this problem can be reduced by the collection of RBC components by apheresis.<sup>6</sup>

The second problem is RBC cryopreservation. Unless a modified method described by Meryman and Hornblower<sup>7</sup> is used, the deglycerolization process exposes RBCs to hyperosmotic saline, which produces extreme hemolysis of sickle cell trait donor RBCs and yields a dark red, jelly-like mass rather than a suspension of RBCs. The standard deglycerolization procedure used with a semiautomatic cell washer involves dilution of the thawed cells with 12% saline followed by dilution with 1.6% saline and washing with 0.8% saline plus 0.2% glucose.<sup>8</sup> The modified procedure involves dilution of the thawed cells in 12% saline followed by dilution in a large volume of 0.8% saline plus 0.2% glucose followed by washing in 0.8% saline plus 0.2% glucose.<sup>7</sup>

The Meryman and Hornblower method,<sup>7</sup> however, was described for use with traditional processing, which involves an open system. When an open system is used, once the RBC components are thawed and deglycerolized, they expire within 24 hours. This short shelf-life can result in the loss of RBC components with rare phenotypes if they are unable to be transfused within 24 hours. However, allowances do exist to refreeze rare donor units one time.<sup>2</sup>

Recently, the FDA has approved an automated blood processing system, ACP 215, to freeze and deglycerolize RBC components.<sup>9,10</sup> This instrument is a functionally closed system conferring a 14-day outdate on the component. In addition, deglycerolization of RBCs with the automated blood-processing system involves dilution in 12% saline and washings in 0.9% saline plus 0.2% glucose.<sup>9,10</sup> Inasmuch as these deglycerolization solutions were similar to those described by Meryman and Hornblower,<sup>7</sup> we hypothesized that the ACP 215 may allow for the successful deglycerolization of sickle cell trait donor RBC components. This study assessed the feasibility of using the ACP 215 for the cryopreservation and deglycerolization of RBC components from donors with sickle cell trait.

## Materials and Methods

### *Study Design*

Donors with sickle cell trait were recruited from a population of healthy people who regularly donate blood for research studies at our institution, and informed consent was obtained before the blood was collected. Whole blood units were collected and processed into nonleukoreduced RBC components from three donors with sickle cell trait, and each component was processed with an automated cell-processing system (ACP 215, Haemonetics Corp., Braintree, MA) and cryopreserved within 6 days of collection. Although the ACP 215 is not approved for processing CP2D/AS-3 nonleukoreduced RBCs, leukoreduction was not performed in this study to avoid filter failure and loss of sickle trait units. The components were stored at  $-65^{\circ}\text{C}$  or less for at least 2 days and were deglycerolized using the ACP 215's standard procedure. Before cryopreservation and after deglycerolization, several variables were measured including weight, hematocrit, and supernatant hemoglobin levels. Data from RBC components collected from six healthy routine blood donors without

sickle cell trait were collected and used as controls. These control units were cryopreserved, thawed, and deglycerolized using the same automated cell-processing system. All the donors met the AABB criteria for donating whole blood. The study was approved by the institutional review board.

### *Study Population*

Blood from three donors with sickle cell trait was studied. Donor 1 was a 23-year-old woman with a mixed ethnic background. Donor 2 was a 59-year-old Black woman. Donor 3 was a 37-year-old Black woman. The control units were collected from healthy donors ranging from 50 to 61 years of age. Four of the control donors were men and two were women. All were Caucasian.

### *Collection and Processing of RBC Units*

A unit of whole blood was collected using a standard collection method for whole blood drawn into a CP2D/AS-3 triple blood bag set with an in-line filter and Y sampling site (Pall Corporation, East Hill, NY). Within 4 hours of collection, the whole blood was spun down and plasma was expressed off the unit.

The in-line leukocyte filter was then removed using a heat sealer (Composeal Mobilea, Fresenius HemoCare, Redmond, WA), and the tubing was re-joined to the bag containing the AS-3 using a sterile connecting device (SCD sterile tubing welder, Terumo Medical Corporation, Somerset, NJ). Next, the AS-3 was added to the packed RBCs without leukocyte filtration. The RBC component was then refrigerated at 1° to 6°C for at least 2 days before freezing.

### *Cryopreservation*

The AS-3 RBC components were centrifuged and the supernatant removed. The units were then sterilely connected to the ACP 215 standard disposable glycerolization kit (Haemonetics REF 225, ACP 215 Red Cells Glycerolization Disposable Set, Haemonetics Corp.), and were glycerolized automatically by the ACP 215 according to manufacturer's procedure. Once glycerolized, the units were centrifuged and excess glycerol was expressed off. The components were heat sealed in a polyester plastic bag to maintain sterility and were frozen. The units were stored for at least 2 days at -65°C or less before deglycerolizing. A more comprehensive description of the process using the ACP 215 can be found in a study by Valeri et al.<sup>9,10</sup>

### *Deglycerolization*

The units were removed from the freezer and placed into a 37°C water bath until they reached a temperature of 32° ± 2°C. Using the ACP 215 disposable deglycerolization set (Haemonetics REF 235, ACP 215 Red Cells De-Glycerolization and Cell Wash Disposable Set, Haemonetics Corp.), the components were deglycerolized first with 12% saline followed by multiple large-volume washes using 0.9% saline containing 0.2% glucose. After washing was complete, the final product was resuspended in AS-3. In-process pressure checks at the end of deglycerolization indicated that the system remained closed and the units could retain a 14-day outdate. As an additional indication of acceptability, a visual hemolysis check was performed on the waste supernatant using a color chart provided with the ACP 215 to ensure the free hemoglobin levels were less than 150 mg/dL. A sample was taken for analysis of several variables including analysis of the wash supernatant for plasma hemoglobin and osmolality.

### *Laboratory Analysis*

At the time of the collection of the unit of whole blood, an additional tube of blood was drawn into EDTA for HbS analysis. HbS was analyzed using ion-exchange high-performance liquid chromatography (Varian HPLC system, Bio-Rad Diagnostics Group, Hercules, CA).

Before cryopreservation and after deglycerolization of the RBC component, samples were taken for laboratory analysis and components were weighed for the calculation of the percent recovery of RBCs. The



**Fig. 1.** A dark red, jelly-like mass in the bowl after deglycerolization.



**Table 1.** Results of in vitro tests of RBC components before and after deglycerolization with the ACP 215

Donor	Hct (%)		HbS (%)	RBC Recovery (%)	Supernatant Hgb (mg/dL)		Color Check (<150 mg/dL)	Osmolality (mOsm/kg)
	Before	After			Before	After		
1	64	26	34	43	150	1200	No	222
2	67	47	38	76.5	130	480	Yes	212
3	62	40	39	67.5	120	460	Yes	315

blood sample was used to test the hematocrit using a microhematocrit system (HemataStat II, Separation Technologies, Inc., Altamonte Springs, FL). Plasma hemoglobin levels were also analyzed with a low-Hb photometer (HemoCue AB, Angelholm, Sweden). An iSTAT (i-STAT Corporation, East Windsor, NJ) was used for measurement of blood gas analytes.

In addition, samples of the wash supernatant were analyzed for osmolality to ensure adequate removal of the glycerol. Osmolality was performed using the method of freezing point depression on a microsample osmometer (Fiske 2400, Advanced Instruments Inc., Norwood, MA).

### Statistics

Because of the relatively small sample size ( $n = 3$ ), statistical analysis was not performed as significance could not be determined.

### Results

Thawing and deglycerolization of all three RBC components from the donors with sickle cell trait resulted in the recovery of some RBCs (Table 1); however, the plasma hemoglobin level on the post-deglycerolization supernatant sample from Donor 1 was extremely high. All components had acceptable osmolality levels of less than 400 mOsm/kg, indicating that glycerol removal was adequate. Only the RBC component from Donor 2 passed the in-process pressure check indicating that the system remained closed during processing, conferring a 14-day out-date. A dark red, jelly-like mass was noted in the bowl after deglycerolization of all three components and was the largest for the component from Donor 1 (Fig. 1).

The hematocrit levels of the deglycerolized components ranged from 26 to 47 percent, and the RBC recoveries ranged from 43 to 76 percent. The donors' HbS levels ranged from 34 to 39 percent. There appeared to be no relationship between RBC recovery and the donors' HbS levels (Table 1). As expected, glucose and potassium levels in the RBC components decreased after deglycerolization and sodium levels

**Table 2.** Results of analysis of sickle cell trait RBC components before and after deglycerolization with the ACP 215

Donor	Sodium (mmol/L)		Potassium (mmol/L)		Glucose (mg/dL)	
	Before	After	Before	After	Before	After
1	118	131	>9.0	2.8	681	569
2	122	130	>9.0	<2.0	662	542
3	121	132	>9.0	<2.0	676	508

**Table 3.** Results of analysis of RBC components from donors without sickle cell trait that were cryopreserved and deglycerolized with the ACP 215

Donor	Osmolality (mOsm/kg)	RBC Recovery (%)	Hct (%)	Color Check (<150 mg/dL)
1	304	92	52	Yes
2	303	87	52	Yes
3	297	85	52	Yes
4	298	80	49	Yes
5	304	84	51	Yes
6	308	91	52	Yes

increased (Table 2). Washing removes approximately 99 percent of plasma proteins, electrolytes, and antibodies<sup>2</sup> and exchanges residual CP2D for residual 0.9% saline with 0.2% glucose. In comparison, all six control RBC components collected from donors without sickle cell trait had a postdeglycerolization RBC recovery of 80 percent or greater, an osmolality of less than 400 mOsm/kg, and hematocrit levels of 49 percent or greater (Table 3). Glucose, potassium, and sodium were not measured on the control units.

The sickle cell trait donors' RBC components were all frozen 4 or 5 days after their collection and were stored for 4, 35, and 56 days before deglycerolization. RBCs from Donor 1 were cryopreserved the longest followed by Donors 2 and 3. Future studies using the ACP 215 are needed to conclude whether there is a relationship between storage time and percent recovery after deglycerolization.

## Discussion

An automated cell-processing system, APC 215, is available for the freezing and deglycerolization of RBC components and is being used to save rare donor units. We tested this system using the manufacturer's recommended procedures to process RBC components from donors with sickle cell trait to assess the feasibility of obtaining an acceptable cryopreserved and deglycerolized RBC component for transfusion. RBC components from all three donors tested resulted in recovery of some RBCs. However, none of the deglycerolized components met the criteria for greater than 80 percent recovery of RBCs, and congealed RBCs were noted in the bowl after the processing of each of the three components. The process resulted in components that had osmolality levels that indicated the units were successfully deglycerolized, but it is not certain whether the posttransfusion recovery and survival of these components would be normal.

The mean of RBC recovery for our study was 62 percent compared to 56 percent by Meryman and Hornblower<sup>7</sup> using the traditional deglycerolization method and a manual cell washer. The RBC recovery with the ACP 215, however, was not as good as with the modified method for processing sickle cell trait donor RBC components described by Meryman and Hornblower<sup>7</sup>; mean recovery was 85 percent. We do not recommend that the standard ACP 215 cryopreservation and deglycerolization methods be used for the routine processing of sickle cell trait donor RBCs, but it may be possible to modify the deglycerolization procedures used with the ACP 215 method to improve the recovery of sickle cell trait RBCs. Because hemolysis of sickle cell trait donor RBCs occurs in the deglycerolization process,<sup>7</sup> modification of the ACP 215 deglycerolization solution may improve RBC recovery. Perhaps greater dilution of the thawed RBCs with the solution containing 0.9% saline plus 0.2% glucose or increasing the pH of the deglycerolizing solutions<sup>11</sup> would reduce hemolysis. Minimizing osmotic damage during glycerolization by adding glycerol more gradually through the use of additional steps may also be helpful.<sup>12</sup>

In conclusion, freezing and deglycerolization of sickle cell trait donor RBC components with the ACP 215 system resulted in better recovery than did the manual method, but it was not better than that produced by the modified method by Meryman and Hornblower.<sup>7</sup> However, hemolysis and agglutination

in the bowl were problematic. Future modifications of the deglycerolization procedure using the ACP 215 are needed for successful processing of sickle cell donor RBC components.

## References

1. Castro O, Sandler SG, Houston-Yu P, et al. Predicting the effect of transfusing only phenotype-matched RBCs to patients with sickle cell disease: theoretical and practical implications. *Transfusion* 2002;42:684-90.
2. Brecher M. Technical Manual. 15th ed. Bethesda, MD: AABB Press, 2005;576,193.
3. Osby M, Shulman I. Phenotype matching of donor red blood cell units for nonalloimmunized sickle cell disease patients: a survey of 1182 North American laboratories. *Arch Pathol Lab Med* 2005;129:190-3.
4. Beattie KM, Shafer AW. Broadening the base of a rare donor program by targeting minority populations. *Transfusion* 1986;26:401-4.
5. Gorlin JB, Adams CL, Stefan MM, Averbek DA, Gonnella KC. Variable leukocyte reduction on units from donors with sickle cell trait: a time-temperature, donor reproducibility study (abstract). *Transfusion* 2000;40(Suppl):55.
6. Stroncek DF, Rainer T, Sharon V, et al. Sickle Hb polymerization in RBC components from donors with sickle cell trait prevents effective WBC reduction by filtration. *Transfusion* 2002;42:1466-72.
7. Meryman HT, Hornblower M. Freezing and deglycerolizing sickle-trait red blood cells. *Transfusion* 1976;16:627-32.
8. Meryman HT, Hornblower M. A method for freezing and washing red blood cells using a high glycerol concentration. *Transfusion* 1972;12:145-56.
9. Valeri CR, Ragno G, Pivacek LE, et al. A multicenter study of in vitro and in vivo values in human RBCs frozen with 40-percent (wt/vol) glycerol and stored after deglycerolization for 15 days at 4 degrees C in AS-3: assessment of RBC processing in the ACP 215. *Transfusion* 2001;41:933-9.
10. Valeri CR, Ragno G, Pivacek L, et al. In vivo survival of apheresis RBCs, frozen with 40-percent (wt/vol) glycerol, deglycerolized in the ACP 215, and stored at 4 degrees C in AS-3 for up to 21 days. *Transfusion* 2001;41:928-32.

11. Rivers SL, Liles B. Problems associated with freezing and thawing of red blood cells containing AS hemoglobin (abstract). *Transfusion* 1973;13:356.
12. Valeri CR, Lindberg JR, Contreras TJ, et al. Freeze preserved baboon red blood cells: effects of biochemical modification and perfusion in vitro. *Am J Vet Res* 1981;42:1590-4.

---

*Ricci Jo Ackley, MT(ASCP)SBB, Interim Manager, George Washington University Hospital, Transfusion Medicine, Washington, DC, A. Hallie Lee-Stroka, MT(ASCP)SBB, Technical Specialist, National*

*Institutes of Health, Department of Transfusion Medicine, Clinical Center, Barbara J. Bryant, MD, MT(ASCP)SBB, Staff Physician, National Institutes of Health, Department of Transfusion Medicine, Clinical Center, David F. Stroncek, MD, Chief, Laboratory Services Section, National Institutes of Health, Department of Transfusion Medicine, Clinical Center, and Karen M. Byrne, MDE, MT(ASCP)SBB, (corresponding author) Education Coordinator, National Institutes of Health, Department of Transfusion Medicine, Clinical Center, 10 Center Drive MSC 1184, Bethesda, MD 20892-1184.*

**Notice to Readers:** All articles published, including communications and book reviews, reflect the opinions of the authors and do not necessarily reflect the official policy of the American Red Cross.

**Attention: State Blood Bank Meeting Organizers**

If you are planning a state meeting and would like copies of *Immunohematology* for distribution, please **contact** Cindy Flickinger, Managing Editor, 4 months in advance, by fax or e-mail at (215) 451-2538 or flickingerc@usa.redcross.org.